Chromosomal Structures in Crayfish Spermatocytes.*

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To date the electron microscope has yielded little about the orderly macromolecular structure of chromosomes that, from genetical and cytological evidence, might be expected. Indeed, even the grosser morphology long familiar to cytologists in the light microscope is harldy recognizable, partly for want of suitable electron stains and partly, undoubtedly, because of fixation and preparative effects and the complexity of interpreting structures in thin sections. It is true that refined methods of preparing tissues for study in the electron microscope—particularly those of buffered osmium tetroxide fixation, plastic embedding, and thin sectioning—have vastly improved the quality of material studied, but the cytology of the nucleus at the electron microscope level of resolution remains obscure.

Most consistent are recent reports of coiled structures about 500 A wide seen in isolated chromosomes (whole (1) and in sections (2)) and areas assumed to be occupied by them *in situ*. These have been reported as being comprised of paired strands about 200 A wide (3) but there is difficulty and hence disagreement in interpreting them as filaments or granules (4). While such entities may well prove to be morphological subunits of the chromosome, at the moment they present a disappointingly random aspect. Orderly organization intermediate to them and the coarser details such as chromonemata (at the limit of light resolution) and gyres (best visible in specially treated material) seen in the light microscope is lacking.

Details of the latter structures are most apparent at stages in the cell cycle such as meiotic prophase where the chromosomes have emerged as easily discernible entities and have not yet undergone extensive coiling and condensation prior to metakinesis. Light microscopists have derived much of our present knowledge of chromosomes from studies of such stages. It is here also that we may expect to find structure with the electron microscope. Watson (5), for example, has reported fine paired filaments less than 400 A wide in primary spermatocyte prophases in the rat. These may be chromosomal elements, the counterparts of which we have observed in spermatocytes of grasshopper and crayfish. In the latter the condition is sufficiently striking to warrant a brief description.

Testes from mature *Cambarus clarkii*[1] were fixed for 1 hour in veronal-acetate buffered 1 per cent OsO_4 at pH 8 and 8.4. The meiotic chromosome cytology, which has been described by Fasten (6) was followed in the light microscope. The large number of chromosomes (*ca.* 100) together with their characteristic polarization and attachment to the nuclear periphery raises the probability of including aspects of various planes through chromosome axes in a thin section.

In the electron microscope, chromosomes are about 1 μ wide, depending on

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stage and chromosome region. A central dense region (Fig. 1, *C*) about 150 mμ in width follows the chromosome axis and is clearly visible at low powers. Fig. 1 shows a characteristic part of a primary spermatocyte prophase chromosome (*CR*) with one end closely applied to the double nuclear envelope (*NE*). A second chromosome passes out of the section just below it. The chromosome profile is clear and of some interest, but will not be discussed here. Such structures are Feulgen-positive when studied by light microscopy in adjacent thick sections. The chromosome lies parallel to the plane of section for a good part of its length and has been cut so that the section passes through the central dense region. The latter can be seen to follow the bend in the chromosome and otherwise appears to be an integral part of it. A section of the same chromosome is shown in Fig. 3 at higher magnification. The interior of the dense region gives the appearance of a series of alternate parallel light and dark lines. They cannot be followed to the nuclear envelope, probably because of curving of the chromosome. Such parallel arrays in the dense region are commonly visible in prophase chromosomes of crayfish, though seldom at such length. Fig. 2 is a section from another cell that includes a similar longitudinal cut. It typifies our most consistent observation, that of a continuous dense central structure (*P*) less than 150 A wide bounded on either side by less dense areas about 250 A wide (*Q*). These are in turn bounded by one or more roughly parallel 150 *A* lines (*R*), frequently discontinuous and separated by varying distances. The characteristically non-homogeneous substance of the remainder of the chromosome blends with the dense area at its periphery, occasionally appearing to be arranged in parallel array perpendicular to the central apparatus. Fig. 4 presents three aspects of increasing degrees of obliquity (*a*, *b*, *c*), approaching the normal to the longitudinal axis of the chromosome in a thicker section. The appearance is consistent with the central structure or "core" (*C*, Figs. 1 and 2) being composed of a median dense rod embedded in a less dense material. This in turn is surrounded by varying numbers of concentric dense shells which become less distinct progressing radially. Fig. 5 is a very thin section showing cross (*X*) and longitudinal (*L*) sections of the "core." While the cross-section gives the impression of several concentric circles, it can be seen to be actually formed by imperfect aggregations of finely divided discontinuous material. The longitudinal section has a corresponding appearance with the rod and its shell being most nearly perfect. Almost every prophase chromosome observed to be cut through its axis has shown some evidence of this "core." Late prophase and metaphase chromosomes lack it entirely. We have not yet discovered its origin or followed it through sequential stages.

Chromosomal "cores" showing substantially the same detail have been occasionally encountered in other materials. However, our observations have so far been limited and it cannot be said if the structure reported here is common to all chromosomes. It is realized that our present knowledge of chromosomes based on observations with the light microscope does not lead us to expect the structures described. However, they may well represent either a special or transient form of a fundamental structure whose role in chromosome function is less obscure.

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EXPLANATION OF PLATE 36

All chromosomes are from primary spermatocyte prophases of the crayfish. Bars denote approximately 100 mμ.

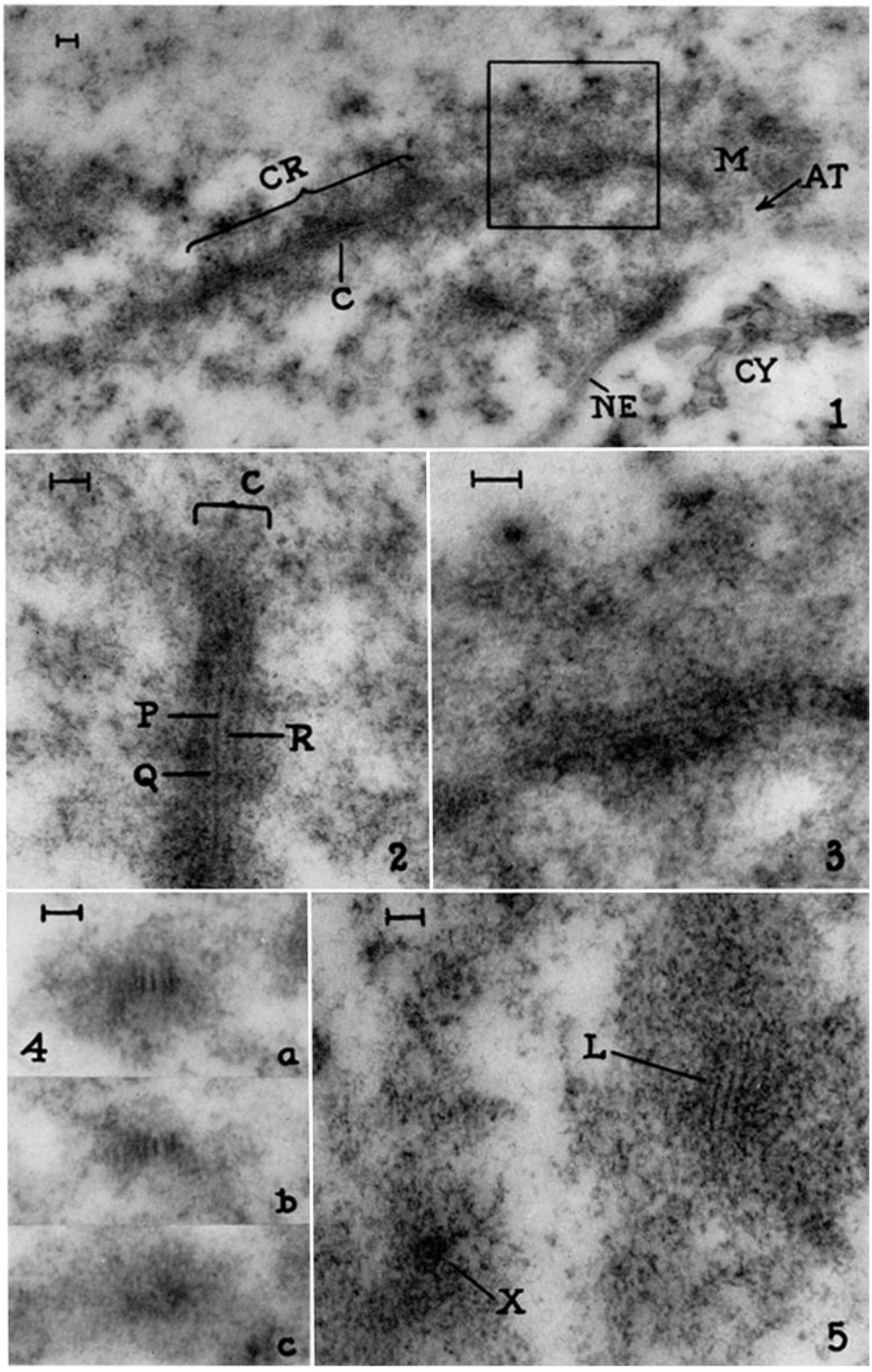
FIG. 1. Longitudinal section of chromosome (*CR*) cut along its axis. *C*, central dense region; *CY*, cytoplasm; *NE*, nuclear envelope; *AT*, region of chromsome association with nuclear envelope (membranes are cut obliquely at this point); *M*, discontinuous chromosomal material. Approximately × 25,000.

FIG. 2. Longitudinal section through axis of chromosome showing details of structure in central dense region (*C*). *P*, *Q*, *R*, parallel structures described in text. Approximately × 56,400.

FIG. 3. Higher magnification of portion of chromosome outlined in Fig. 1. Approximately × 77,000.

FIG. 4. Three oblique sections through chromosome central region, approaching the normal and the axis at *c*. Approximately × 56,400.

FIG. 5. Cross (*X*) and longitudinal (*L*) sections of chromosome "cores" seen in very thin section. Approximately × 56,400.



(Moses: Chromosomal structures in spermatocytes)